

Cytogenetic Biomonitoring in Buccal Mucosa Cells of COVID-19 Patients: Preliminary Findings

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Abstract. *Background/Aim:* COVID-19 may lead to progressive respiratory failure as a consequence of alveolar damage, resulting in death. The aim of this study was to evaluate cytogenetic damage in oral cells of COVID-19 patients by micronucleus assay. *Patients and Methods:* A total of 11 COVID-19 patients aged 40.7 ± 9.3 years (5 men and 6 women) were included in this study. For the control group, a total of 15 participants not infected with SARS-CoV-2 virus were included. The mean age was 41.6 ± 6.2 years (5 men and 10 women). *Results:* The results showed statistically significant differences ($p < 0.05$) in micronucleated buccal mucosa cells of COVID-19 patients. In addition, a statistically significant increase in karyolysis and karyorrhexis ($p < 0.05$) was observed in COVID-19 patients compared to control. *Conclusion:* SARS-CoV-2 virus can induce mutagenesis and cytotoxicity in oral cells.

A great number of patients were diagnosed with pneumonia in Wuhan, Hubei province, China in the late last year. After proper investigation, it was concluded that pneumonia was caused by a novel coronavirus named SARS-CoV-2, and the disease was called COVID-19 (1). The most common symptoms characterizing COVID-19 are fever followed by, dry cough, fatigue, prostration, myalgia, and dyspnea (2).

Unfortunately, it has been verified that COVID-19 may lead to progressive respiratory failure as a consequence of alveolar damage, resulting in death (1). In an earlier study conducted by Zhou *et al.* (1), it was demonstrated that the

angiotensin-converting enzyme II (ACE2) is the exclusive cell receptor for SARS-CoV-2 in human cells. Furthermore, Xu *et al.* have revealed that the RBD domain of SARS-CoV-2 demonstrates close interaction with human ACE2 receptors (3). Taken together, these findings suggest that ACE2 receptors play a pivotal role in the molecular mechanisms underlying the pathogenesis of SARS-CoV-2 infection. Therefore, the expression as well as the distribution of the ACE2 receptors in human cells and/or tissues may indicate important infection routes of SARS-CoV-2.

It has been hypothesized that ACE2 receptors are present in oral tissues, such as the tongue mucosa, buccal cells, and gingival tissue (2). A previous study conducted by Nakamura *et al.* (2) has postulated that ACE2 receptors are expressed in gingival fibroblasts *in vitro*. These results are consistent with the fact that other cellular types present in the gingival tissue may express ACE2 receptors as well (4).

It is well established that genetic damage is responsible for genomic instability that underlies the development of many chronic degenerative diseases. In infectious diseases, micronuclei have been identified as useful biomarkers of cell damage (5). Micronuclei indicate biological events closely associated with genotoxicity allowing for a better understanding of the etiopathogenesis and consequently, for proposing prevention strategies (6). Thus, the identification of micronucleated cells in the oral mucosa of COVID-19 patients may be a useful tool to clarify the biological effects of SARS-CoV-2 in epithelial tissues, such as oral mucosa cells.

Thus, the aim of this study was to evaluate cytogenetic damage in oral cells from COVID-19 patients.

Patients and Methods

Participants. A total of 11 COVID-19 patients aged 40.7 ± 9.3 years (5 men and 6 women) were included in this study. The volunteers were selected from the Center of Emergency and Hospitalization in the São Vicente city, SP, Brazil. For the control group, a total of 15 not infected participants aged 41.6 ± 6.2 years (5 men and 10 women) were included in this study. All volunteers were diagnosed with COVID-19 by Real time PCR (SARS-CoV-2 Virus detection) or One Step Rapid Test Covid-19 IgG/IgM. A total of two individuals used

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azithromycin and ivermectin in the experimental group. All individuals (control and experimental groups) were non-smokers, except for one person in the experimental group. Furthermore, no exposure to dental X-ray was monitored in the last month. In addition, no oral lesion was visible at clinical evaluation. However, alcohol consumption was not recorded. All demographic characteristics of the participants of the study are summarized in Table I. The study was approved by the Ethics Committee of the Federal University of São Paulo, UNIFESP, number #1448/2020. Informed consent was signed by all individuals included in the study.

Micronucleus test on oral mucosal cells. The micronucleus test was performed according to the method described by Andrade *et al.* (7). For this purpose, exfoliated oral cells from all volunteers were collected. This was achieved by scrapping the right/left cheek mucosa with a moist wooden spatula. After that, oral cells were transferred to falcon tubes containing saline solution, centrifuged (800 rpm) for 5 min, fixed in 3:1 methanol/acetic acid, and spread over glass slide. Later, all slides were stained with the Feulgen/Fast Green method.

Data analysis. All slides were blindly evaluated by using a light microscope at $\times 1000$ magnification to identify the presence of micronucleated cells and metanuclear alterations indicative of cytotoxicity. Micronuclei were scored according to the criteria described by Belien *et al.* (8) as a parameter of DNA damage (mutagenicity). For cytotoxicity, the following nuclear alterations were considered: pyknosis, karyolysis, and karyorrhexis. Results were expressed as a percentage of total cells examined. This analysis was established in a previous study conducted by our research group (8). A total of 2,000 cells were evaluated per volunteer.

Statistical methods. The Student's *t*-test was used to compare the frequencies of micronucleus and cytotoxicity between the experimental group *versus* the control group (9). The statistical analysis was conducted using BioStat software, version 5.0 (Maringá, PR, Brazil). The level of statistical significance was set at 5%.

Results

The results showed statistically significant differences ($p < 0.05$) in micronucleated cells in buccal mucosa cells of COVID-19 patients (Figure 1D, Table II).

When cytotoxicity parameters were evaluated, interesting results were observed. First, pyknosis was not different between the COVID-19 group and the control group (Figure 1A). Nevertheless, karyolysis and karyorrhexis were statistically increased in the COVID-19 group compared to the control group ($p < 0.05$) (Figure 1B and C, Table II).

Discussion

The aim of this study was to evaluate cytogenetic damage and cell death in buccal mucosa cells from COVID-19 patients as indicators of chromosomal injury and cytotoxicity, respectively. Evaluation was performed using the micronucleus test in buccal cells. To the best of our knowledge, this question has not been previously addressed.

Table I. Demographic characteristics of the participants of the study.

Parameters investigated	Control group (n=15)	COVID-19 patients (n=11)
Mean age	41.6 \pm 6.2	40.7 \pm 9.3
Gender (Male/Female)	5/10	5/6
Medicines	-	2 (azithromycin and ivermectin)
Dental X-ray	-	-
Mouthrinse	-	1
Smoking	-	1
Illicit drugs	-	-
Chemo- or Radiotherapy	-	-

SARS-CoV-2 virus is a member of the coronavirus family and affects humans (10). The coronavirus has a simple structure consisting of several structural proteins, such as the envelope protein (E), spike protein (S), transmembrane protein (M), and nucleoprotein (N) (11). Except for the N protein, the E, S, and M proteins promote virus entry into the mammalian cells, and result in viral pathogenesis (10).

To date, there are direct and indirect ways of transmission for SARS-CoV-2. Direct transmission comprises contact with the infected individual's blood, salivary or respiratory droplets, as well as the urine, feces, semen, and tears (12). The signs and symptoms of COVID-19 are categorized into respiratory and systemic manifestations. The most common are fever, cough, and fatigue (13, 14). However, there are further manifestations, such as oral mucosal lesions and neurological disorders, for example loss of smell and taste, myofascial pain, and headache. These symptoms have been included in the diagnostic criteria for COVID-19. The oro- and nasopharynx, and the nasal cavities are potential sites for SARS-CoV-2 virus replication (15, 16). For this reason, it is interesting to examine if, and to what extent, SARS-CoV-2 virus is able to induce mutagenesis and/or cytotoxicity in oral cells *in vivo*. Our results indicated high frequencies of micronucleated cells in buccal mucosa cells of COVID-19 patients when compared to the control group. Herein, it is important to highlight that the micronucleus is induced by chromosome breakage or loss. Therefore, the presence of this cytogenetic parameter indicates mutagenicity in epithelial cells as a result of genomic instability. However, the consequences of this situation are unclear and therefore, they must be investigated.

In order to better elucidate the mechanisms by which SARS-CoV-2 virus modulates the biological machinery involved cell death, cytotoxicity was also evaluated. Among the parameters chosen for this purpose, pyknosis did not present significant differences between the infected and healthy control groups. However, our results demonstrated a significant increase in karyorrhexis and karyolysis in the buccal mucosa of COVID-19 patients. These results are novel, and therefore, a subject for

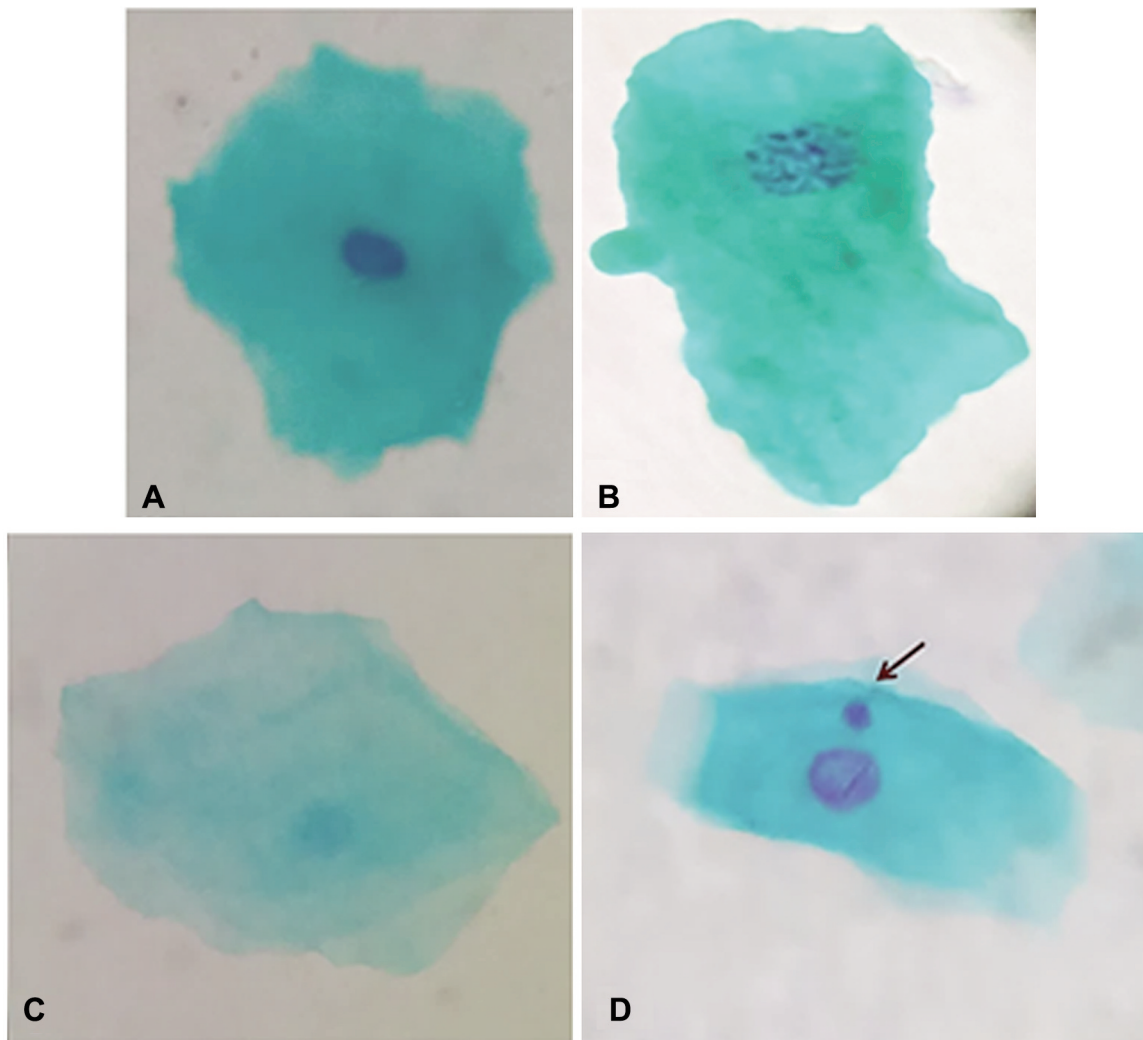


Figure 1. Metanuclear changes in buccal mucosa cells of COVID-19 patients. A: Pyknosis, B: Karrhyorexis; C: Karyolysis, and D: micronucleated cell (arrow). 100 \times magnification. Feulguen-Fast-Green stain.

Table II. Mean and standard deviation (% of cells) of cytogenetic parameters (micronucleus, pyknosis, karrhyorexis and karyolysis) in mucosa cells of COVID-19 patients and healthy controls.

Groups	Micronucleus	Pyknosis	Karrhyorexis	Karyolysis
Control (n=15)	0.2 \pm 0.5	132.6 \pm 54.6	23.8 \pm 7.9	169.4 \pm 60.9
COVID-19 patients (n=11)	1.4 \pm 1.5*	97.2 \pm 46.4	48.9 \pm 15.5*	269.7 \pm 71.3*
p-Value	p=0.01	p=0.11	p<0.001	p=0.01

*p<0.05 when compared to the control group.

scientific discussion. It has been established that karryorhexis is closely associated with apoptosis. This is a relevant biological process, in which cell death is genetically controlled for both normal development and tissue homeostasis (17). The

second finding is that karyolysis is closely associated with necrosis (17). Despite the fact that the biological mechanisms by which this type of cell death is triggered have not been fully elucidated, it has been postulated that necrosis is induced by a

high magnitude insult. Overall, our findings show that the SARS-CoV-2 virus induces cellular death in buccal cells either by necrosis or apoptosis. By comparison, Haga *et al.* postulated that SARS-CoV viruses induce TNF- α expression (18). TNF- α is an inflammatory cytokine produced by mononuclear cells during acute inflammation and is responsible for a diverse range of cellular signaling pathways, leading to necrosis or apoptosis (19).

We hypothesized that cellular pathways activated by the interaction of SARS-CoV with ACE2 are involved in viral entry and tissue injury. In fact, oral viral infections are common in clinical practice that are associated with oral lesions. Earlier studies have published some oral symptoms induced by SARS-CoV-2 infection, as for example ulceration and vesicular bullous lesions (20, 21). In the oral mucosa, viral infections damage epithelial cells causing acute inflammatory response, presenting with solitary and multiple blisters or even ulcerations (22). Microscopic analysis of biopsies from COVID-19 patients who also presented skin lesions confirmed the vascular ectasia associated with congested vessels, and lymphocytic inflammatory infiltrate (23). Taken together, high ACE2 expression is detected in some cellular types, such as alveolar cells, oropharyngeal mucosa, kidney, gastrointestinal tract, endothelial cells, and oral tissues (16). This finding suggests that such organs and/or tissues with high ACE2-expressing cells must be evaluated individually as much as possible since they are high-risk sites for SARS-CoV-2 infection, especially for mutagenicity and cytotoxicity.

In conclusion, the results of the present study suggest that SARS-CoV-2 virus is able to induce mutagenesis and cytotoxicity in oral cells. However, further studies are needed to better understand whether SARS-CoV-2 virus promotes mutagenesis associated or not with cytotoxicity in other sites such as the gingiva, tongue, and nasal cells. This information will contribute to the better understanding of the disease as well as the development and authentication of oral diagnostics for COVID-19.

Conflicts of Interest

All Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Conceptualization: MESA and DAR. Data search: TGP and MESA. Formal analysis: TGP, MESA, and DAR. Writing - review & editing: TGP, MESA, and DAR.

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